There are 2 days to the lab

PART 1: prepare samples **1 hr** (this could be done in advance and frozen – i.e. previous day)

Turn on 95deg Heat block first thing.

PART 2: load and run gel (**1 hr)** and stain gel (**1 hr)**

Destain **overnight**

PART3: Image destained gel (either 1, 2 or 3 days after Part 2)

Dry gel on dryer and take another image (**2 hrs)**

**REAGENTS:**

The Blue loading dye: use the one for protein, not the DNA loading dye! The glycerol will make it sticky – so they should cut their pipette tip with scissors if necessary.

We have both Imperial Protein stain that is **used** (‘recycled’) and **fresh** – both work fine.

* they need to save it when they are done (don’t dump Imperial Protein stain in the drain)

**IMPORTANT CONCEPTS & SKILLS:**

**You will have to demonstrate how to clear out the wells with a syringe first (then let them do the rest)**

**You will really have to watch them load their samples.**

**When there gel is done running – they won’t be able to see any bands (only the ladder) – ask them what was wrong with their gel? They will freak out – but when they stain it – they will see their bands within an hour!**

* Those bigger proteins will be at the top of the gel
* This is a denaturing gel – proteins are NOT in the native conformation – but rather unraveled

Explain the different lanes to them and what they ‘should’ see

**Ladder** – comes from proteins of known sizes that have been ‘stained’ pretty colors!

**Cell lysate** (sample 1) – a bunch of junk- or won’t even run

**Soluble fraction** (sample 2) – lots of protein (including a big blob for Purple Protein)

**Flow Through** (sample 3)– lots of unbound soluble protein (many bands or a smear)

**Wash** (sample 4) – should have a some proteins show up that were moderately bound

**Elution 1** (sample 5)– ideally – one sharp band that is their Purple Protein

**Elution 2** (sample 6)– no bands or very faint if carry over from Elution 1

Q: How are proteins separated on the gel – by charge or by size?

Q: Different proteins have different charges – why don’t they some go in the opposite direction?

Be sure to help them see the relationship between their Nanodrop readings from the previous lab and their lanes on the gel. They should see a correlation (ie – if their band is strong – they should have a high protein concentration reading from Nanodrop)

Drying the gel – we are basically removing the water and leaving the polyacrylamide mesh behind.

**TROUBLESHOOTING:**

Everything

They do have the ONLINE lab report on WIKISPACES due

Be sure the post take and **post** their **pictures** of the **gel** online

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